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Crown Symptoms of Regrowth Dieback[1]

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ABSTRACT: Symptoms in the crowns of *Eucalyptus obliqua* and *E. regnans* trees affected by "regrowth dieback" are reevaluated and amplified using tree climbing, tree felling, and optical study methods. A distinction is made between the dying upper branches and the lower crown of dieback-affected trees. Declining branches showed an increase in mortality or absence of growing shoots, naked buds, and accessory buds with increase in dieback severity. Mean distance between leaf scars indicated that growth rate of shoots was slowed before death. With increase in dieback severity of a branch, leaf size and leaf area index were reduced. Leaves on dieback-affected branches showed increased chlorosis and reddish colors. There are common features linking dieback-affected shoots with shoots showing mild "witches brooming." Twig-inhabiting insects are considered of only secondary importance. There was no evidence of any culturable microorganism from the vascular system. The symptoms could be the result of water deficits and high leaf temperatures. Alternatively, the symptoms are suggestive that microbes such as a virus or more especially a mycoplasma are involved.

THE DISEASE CALLED "REGROWTH DIEBACK" is an economically important decline of *Eucalyptus obliqua* L'Herit and *E. regnans* F. Muell regrowth trees, especially in southeastern Tasmania (West and Podger 1980). A disease is usefully described from both a population and broad ecological basis as well as from a detailed study of individual plants (Horsfall and Dimond 1959). Podger et al. (1980) have detailed the population and broad ecological relationships of regrowth dieback but provide limited detail on crown symptoms of individual diseased trees. Previous reports (Kile, Turnbull, and Podger 1981, Podger et al. 1980) state that leaf size and color was not affected by the disease.

During physiological work, which involved climbing into the crowns of a few diseased trees (35 m tall), it was evident that these particular trees had noticeably smaller leaves, often chlorotic, on branches that showed declining vigor and dieback.

An initial step in general disease diagnosis is the recognition of symptoms of abnormality evident in diseased individuals (Chester 1959). The correct, detailed diagnosis of symptoms is of critical importance because they constitute the physical abnormalities that require explanation. The symptoms may also provide clues about possible causal factors that could be gainfully examined (Heald 1943). A detailed study of the symptoms of regrowth dieback appears warranted because of the observations about changes in leaf size and color and also because there is no single convincing hypothesis for the cause of this disease (Jehne 1976, Podger et al. 1980).

METHODS

The area chosen for this study was in typical regrowth dieback forests close to Hastings Road, Chestermans Road, and Hay Road, south of Dover in southern Tasmania. These study areas are within 7 km of longitude 146°56′ E and latitude 43°23′ S.

Trees of *Eucalyptus obliqua* and *E. regnans* affected by regrowth dieback are tall (usually over 30 m) and detailed crown examination is

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therefore not simple. Trees were felled by previous workers (Podger et al. 1980), which invariably resulted in breakage and damage especially to the distal part of the crown. This damage is especially severe in the dead and brittle branches of dieback-affected trees. These difficulties were partly overcome by climbing a few diseased trees using ladders or tree-climbing spurs. Scaffolding was erected within the crown of two trees to provide easy access. This allowed nondestructive observations on the progress of symptom development of dying branches. A tripod-mounted binocular (7 × 50) and telescope with magnification up to 60 × was also employed to examine the crowns of other diseased trees. Sketches of the patterns of crown dieback were made, and 14 trees were felled and destructively sampled. Three of the felled trees were diagnosed healthy, while 11 showed varying degrees of dieback. Of the 14 trees felled, 9 were *E. obliqua* and 5 were *E. regnans.*

(1) Distribution of Symptoms within Crowns

A description of the crown position where dieback was first evident was made for 122 trees showing initial signs of dieback. Tree selection was subjectively made from stands within 50 m on either side of Chestermans Road. Crowns were studied with a pair of binoculars and the worst affected branch was recorded as either being the top of the tree, within 3 m of the tree top, or below this point. Also, the worst affected branch was recorded as being within one of the following compass sectors: 0–90°, 90–180°, 180–270°, or 270–360° from magnetic north.

(2) Symptoms of Diseased Branches

Three *Eucalyptus obliqua* and two *E. regnans* trees showing typical early signs of dieback were selected from an area where felling was possible with minimal crown damage (e.g., at edges of recent logging coupes). Three healthy trees were also felled adjacent to diseased trees. The topmost branch of each tree was located, and representative twigs were sampled. These twigs were examined for the presence of living growing tips, naked buds, and accessory buds [following the terminology of Jacobs (1955)]. Accessory buds were examined and dissected using a stereoscopic microscope. For dieback-affected trees, 20 dead twigs were measured for mean distance between the last 10 leaf scars. For healthy trees, 20 typical growing tips were selected and the mean distance between the distal 10 leaf bases determined.

(3) Comparison of Leaves from Healthy and Dieback-Affected Trees

Typical twigs from dieback and healthy trees were selected in three areas as described above. All leaves were harvested and their length, breadth, and areas measured. Leaf area index (L.A.I.) was calculated for each twig as area of leaf per unit area of land surface (Slatyer 1967). Leaves were placed on a light table to assess presence of chlorosis and any other apparent color changes.

(4) Twig Dissection and Pathological Studies

Twenty-seven twigs from declining branches of dieback trees were carefully dissected and color of the phloem and xylem noted as well as any evidence of insects. Surface-sterilized xylem tissue (total of 143 pieces) was plated onto cornmeal agar and incubated at 20°C for up to 26 days. Xylem sap was expressed from chlorotic shoots with a pressure bomb (Ritchie and Hinckley 1975), aseptically collected, streaked onto cornmeal and potato dextrose agar, and incubated at 20°C for up to 26 days. Xylem sap, expressed using the pressure bomb, was microscopically examined at 400 × and 1000 × magnification.

RESULTS

Dieback started at the top of the tree in a majority (94) of the trees, while a further 26 trees showed the worst branch within the upper 3 m of crown, and only 2 trees had the worst affected branch present at a level of 3 m or more below the top of the tree.

The compass bearing of the worst affected branch showed the following numbers of

TABLE 1

MEAN LEAF SIZE AND L.A.I. FOR THREE TWIGS EACH FROM DIEBACK AND HEALTHY TREES

	DIEBACK TREE NO.			HEALTHY TREE NO.		
	1	2	3	1	2	3
Mean leaf length (cm)	8.0	7.2	5.8	10.7	11.2	10.8
Mean leaf breadth (cm)	1.6	1.5	1.2	2.5	2.8	2.5
Mean leaf area (cm^2)	9.2	8.3	4.2	19.5	22.6	19.4
Leaf area index (L.A.I.)	0.36	0.19	0.36	1.69	2.26	1.99

trees in the respective sectors 0–90°, 90–180°, 180–270°, 270–360°: 33, 12, 27, 53. There was a tendency for branch dieback to be initiated in branches on the northern and western sectors of the tree.

The lower branches of dieback-affected trees appeared normal and similar to those of adjacent healthy trees when viewed with binoculars or telescope.

There was a noticeable relationship between an increase in the proportion of dead growing tips and an increase in the dieback severity of any particular branch. Healthy trees had between 0 and 8% of the growing tips dead, while dieback-affected branches commonly displayed 40–60% death of growing tips, even when appreciable numbers of leaves still remained on the twig. Death of growing tips can therefore be considered as an early symptom of dieback.

Naked buds were rarely present on even slightly affected dieback branches. Their previous presence was indicated by a small scar in leaf axils, indicating that they had not developed significantly before abscission.

The presence of accessory buds was demonstrated in over 85% of the leaf axils of twigs collected from the healthy branches of healthy trees. Dieback-affected branches showed reduced numbers of accessory buds with an increase in dieback severity of the branch. Only 12, 15, and 36% of leaves had detectable accessory buds in branches where the L.A.I. was 0.19, 0.23, and 0.36, respectively. These data indicate that the bud system comprising growing tips, naked buds, and accessory buds was increasingly killed with the progress of dieback in an individual branch.

The mean distance between leaf scars on dead shoots ($n = 20$) was 0.52 cm, while for living shoots from healthy trees the mean distance was 1.1 cm; the difference between means was statistically significant at $p = 0.05$. This suggests that extension rates of shoots was reduced for some time before death of shoots occurred.

Mean leaf length, breadth, area, and L.A.I. are shown for each of three twigs from dieback and healthy trees in Table 1. Trees 1 and 3 were *Eucalyptus obliqua* and tree 2 was *E. regnans*.

The difference between means for leaf area of dieback and healthy trees was highly significant ($p < 0.001$). It is clear that leaves are smaller on dieback branches than on healthy branches, and this reduction can become extreme as shown by leaves less than 2 cm long when fully expanded. There is also an obvious reduction in L.A.I. of dieback branches. The mean L.A.I. from Table 1 for healthy branches is a factor of over six times greater than the L.A.I. of dieback branches from dieback trees, and this reflects a leaf thinning of the branch which is considered indicative of incipient dieback.

Leaves from dieback branches frequently showed interveinal chlorosis randomly scattered over the lamina. Chlorosis was especially common in the smaller leaves, particularly leaves less than 5 cm long. Leaves of dieback branches sometimes showed noticeably red coloration in addition to chlorosis. There was seldom any evidence of marginal leaf scorch such as is commonly associated with severe drought (Cremer 1966). The pattern of color changes of leaves on dieback branches was quite similar to those observed in the senescence of leaves due to age on otherwise healthy twigs.

Some dieback trees displayed obvious proliferation of buds in some shoots to produce "witches brooms" (an abnormal proliferation of shoots). Leaves of shoots with severely developed witches brooms showed chlorosis and uneven coloration as well as frequent irregularities in the distribution of oil glands. Upon closer examination, it was observed that many intermediate forms existed between witches brooms and normal shoots. The change to witches brooms could be very rapid, occurring from one leaf axil to the next. Shoots showing mild witches brooming were similar to typical dieback shoots in that both possessed shortened internodes and smaller chlorotic leaves.

Only minor damage to leaves by chrysomelid beetles was observed. However, twigs showed increasing insect damage with decline in vigor of each branch. Partial twig girdling, suggestive of weevils, was common, and insect tunnels about 1 mm in diameter were frequent in diseased twigs of diameter 3–5 mm. An average of 0–0.1 insect tunnel (or cavity) per centimeter was found in healthy twigs, while an average of 0.6 cavity per centimeter was recorded in diseased twigs.

Agar plates indicated that 87% of tissue pieces plated remained free of microbial growth, while the remaining 13% showed a variety of bacteria and an *Alternaria* sp. The streaked sap showed no growth in 11 plates and a bacterial growth in 2 plates. Microscopic examination of expressed sap did not suggest presence of any microbial cells. There is therefore no evidence to suggest that a fungal or bacterial vascular pathogen is involved in the dieback.

DISCUSSION

Regrowth dieback can be interpreted as a branch-by-branch decline, progressing from the top of the tree crown downward. There is no suggestion from the symptoms, culturing, or dissection studies that primary fungal or bacterial infection of branches cause their death. The twig insects found are considered to be of secondary importance only (H. J. Elliott, personal communication).

The decline and death of branches on dieback trees can be viewed as resulting from the death of the bud system followed by accelerated senescence of the remaining leaves which were small in size and frequently yellow and reddish in color. These findings about leaf symptoms of regrowth dieback-affected trees are contrary to those reported by Podger et al. (1980) and Kile, Turnbull, and Podger (1981), who found no evidence of changes in leaf size or color associated with this disease. The author has differentiated between the few declining branches of a diseased tree and the bulk of the lower crown, which was often normal in appearance, whereas Podger et al. (1980) and Kile, Turnbull, and Podger (1981) studied the crown as a whole. This difference in sampling approach is the likely reason for the differences in conclusions about leaf size and color (F. D. Podger and G. A. Kile, personal communication). Also, climbing trees enabled nondestructive observations to be made in the present study, and this was not possible on felled trees which were often badly damaged.

This symptom diagnosis prompts a reconsideration of possible causes for regrowth dieback. Microphylly and chlorosis could conceivably be the result of water deficits in the tree crown (Hsiao 1973). Water deficits cause reduced leaf turgor pressure (Boyer 1968) and reduced growth rates, resulting in smaller and more closely spaced leaves as observed in dying shoots of regrowth dieback-affected trees. Summer water deficits have been measured in the crowns of declining trees which resulted in a calculated loss of turgor and stomatal diffusive resistances of over 20 seconds/cm. High diffusive resistance of leaves significantly increases leaf temperature, thereby increasing the heat load of leaves (Gates and Papian 1971). There were days in these southern forests when air temperature in a Stevenson screen was over 35°C, and on such days temperatures of 42°C have been measured on wilted *Eucalyptus obliqua* leaves. Leaf microphylly, chlorosis, and death of the bud system was possibly initiated by chronic water deficits, which indirectly could also result in leaf overheating and premature senescence (Slatyer 1967). However, this hypothesis is not easily reconciled with observations that large variation in leaf size and color, as well as shoot growth rates, were often evident between adjacent shoots and sometimes within a small portion of a single shoot. Small and chlorotic leaves were not uncommon in the midst of larger healthy leaves on dieback-affected branches. These small leaves are difficult to explain in terms of water and heat stress alone but suggest a biotic agent being of importance. Differential attack by insects, such as already found in twigs, with subsequent effects on the water-conducting capacity of twigs, could be of importance in this context. Studies to investigate the plausibility of this suggestion should be made.

The symptoms described above include microphylly, chlorotic and reddish leaves, and reduced growth rates leading to stunting of shoots. These symptoms are suggestive of other virus diseases in trees (Cooper 1979, Gibbs and Harrison 1980). Podger et al. (1980) briefly considered viruses but their limited work produced no indications of the presence of any virus. The symptoms Podger et al. (1980) perceived in dieback trees were not suggestive of virus disease and contrast with the author's findings. Further study on the possibility of viral involvement in regrowth dieback, using leaves from dying branches, is warranted.

The study of declining shoots suggested they possess features in common with witches brooms. Hence, there is a possibility that witches brooms and typical dieback symptoms share a common causal factor. Virus infection is not known to cause witches brooms, but mycoplasmalike organisms are often implicated (Hull 1971, Seliskar and Wilson 1981). Mycoplasmas, in contrast to viruses, are often lethal to trees, causing a slow decline (Seliskar and Wilson 1981) similar to that observed in trees affected by regrowth dieback. The symptoms noted in the foliage of branches showing dieback are consistent with those of diseases caused by mycoplasmalike organisms, which often produce limited symptom expression in woody hosts (Hull 1971). The possibility of a mycoplasmalike organism being involved in regrowth dieback should be thoroughly investigated.

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